Alterations in Pain Processing Circuitries in Episodic Migraine

Tiffani J. Mungoven
University of Sydney
Kasia K. Marciszewski
The University of Sydney
Vaughan G. Macefield
Baker IDI: Baker Heart and Diabetes Institute
Paul M. Macey
University of California, Los Angeles
Luke Henderson
The University of Sydney School of Medicine https://orcid.org/0000-0002-1026-0151
Noemi Meylakh (noemi.meylakh@sydney.edu.au)
The University of Sydney

Research Article

Keywords: cortical pain modulation, brainstem pain modulation, functional connectivity, PPI, migraine, orofacial pain, dorsolateral prefrontal cortex, hypothalamus, spinal trigeminal nucleus

Posted Date: November 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1029203/v1

Version of Record: A version of this preprint was published at The Journal of Headache and Pain on January 15th, 2022. See the published version at https://doi.org/10.1186/s10194-021-01381-w.

Alterations in pain processing circuitries in episodic migraine

Tiffani J. Mungoven[1], Kasia K. Marciszewski[1], Vaughan G. Macefield[2], Paul M. Macey[3], Luke A. Henderson[1] and Noemi Meylakh[1]

[1]School of Medical Sciences (Neuroscience), Brain and Mind Centre, University of Sydney, Camperdown, NSW, 2050, Australia; [2]Baker Heart and Diabetes Institute, Melbourne, VIC, 3004, Australia; [3]UCLA School of Nursing and Brain Research Institute, University of California, Los Angeles, California 90095, United States.

Corresponding author: Noemi Meylakh, Brain and Mind Centre, University of Sydney, Australia. noemi.meylakh@sydney.edu.au (email), +612 9351 0796 (Tel).

Abstract

Background: The precise underlying mechanisms of migraine remain unknown. Although we have previously shown acute orofacial pain evoked changes within the brainstem of individuals with migraine, we do not know if these brainstem alterations are driven by changes in higher cortical regions. The aim of this investigation is to extend our previous investigation to determine if higher brain centers display altered activation patterns and connectivity in migraineurs during acute orofacial noxious stimuli.

Methods: Functional magnetic resonance imaging was performed in 29 healthy controls and 25 migraineurs during the interictal and immediately (within 24-hours) prior to migraine phases. We assessed activation of higher cortical areas during noxious orofacial stimulation and assessed whole scan and pain-related changes in connectivity.

Results: Despite similar overall pain intensity ratings between all three groups, migraineurs in the group immediately prior to migraine displayed greater activation of the ipsilateral nucleus accumbens, the contralateral ventrolateral prefrontal cortex and two clusters in the dorsolateral prefrontal cortex (dlPFC). Reduced whole scan connectivity dlPFC [Z+44] connectivity with cortical/subcortical and brainstem regions involved in pain modulation such as the putamen and primary motor cortex was demonstrated in migraineurs. Pain-related changes in connectivity of the dlPFC and the hypothalamus immediately prior to migraine was also found to be reduced with brainstem pain modulatory areas such as the rostral ventromedial medulla and dorsolateral pons.

Conclusions: These data reveal that the modulation of brainstem pain modulatory areas by higher cortical regions may be aberrant during pain and these alterations in

this descending pain modulatory pathway manifests exclusively prior to the development of a migraine attack.

Keywords: cortical pain modulation, brainstem pain modulation, functional connectivity, PPI, migraine, orofacial pain, dorsolateral prefrontal cortex, hypothalamus, spinal trigeminal nucleus.

Background

Migraine is a common debilitating neurological disorder, characterized by severe attacks of pulsating head pain with the accompaniment of symptoms such as photophobia, phonophobia, nausea and vomiting. Although the precise underlying mechanisms remain poorly understood, there is growing evidence that changes within the brain itself may be critical for the initiation of a migraine attack (1-3). One emerging hypothesis is that brainstem sensitivity oscillates across the migraine cycle, regulating the brainstem region that receives orofacial noxious afferents: the spinal trigeminal nucleus (SpV). More specifically, altered modulation of the SpV by descending circuits can initiate a migraine by either increasing on-going neural traffic within the SpV or by allowing an external trigger to increase SpV activity; both of which would increase activation of cortical areas and elicit head pain (4).

Consistent with this brainstem oscillation hypothesis, we recently reported that in episodic migraineurs, acute noxious orofacial stimulation evoked greater activation of the SpV compared with controls during the 24-hour period immediately prior to a migraine attack and not during the interictal period (5). This increased activation occurred despite the overall perceived pain intensities being no different to that of the control group (5). In addition, we found that resting state functional connectivity strengths between the SpV and rostral ventromedial medulla (RVM) were reduced only during this same period (5). The most well-described brainstem pain modulatory pathway involves the midbrain periaqueductal gray matter (PAG) - RVM - SpV circuit (6-11) and our results suggest that migraine is associated with fluctuations in descending pain modulatory pathways over the migraine cycle (5).

Brainstem pain modulatory regions are themselves modulated by higher brain regions, as observed by experimental animal investigations, which have revealed that PAG sensitivity is regulated by the hypothalamus and that hypothalamus sensitivity itself is regulated by the cerebral cortex (12-14). One emerging line of evidence is that the hypothalamus is critical for migraine generation (15, 16) and we have previously shown that immediately prior to a migraine, the lateral hypothalamus displayed decreases in resting regional cerebral blood flow and altered connectivity with the PAG, dorsomedial pons, SpV and RVM (17). Whilst we have shown acute orofacial pain evoked changes within the brainstem of individuals with migraine, we do not know if these brainstem alterations are driven by changes in higher brain centers such as the hypothalamus and/or areas of the prefrontal cortex (PFC). It might be that higher cortical and hypothalamic areas may contribute to the initiation and maintenance of migraine pain through their modulation of descending pain modulatory pathways.

The aim of this study is to extend our previous investigation (5) to determine if higher brain centers display altered activation patterns in migraineurs during acute orofacial noxious stimuli. We hypothesize that migraineurs will display altered activation patterns in response to acute orofacial noxious stimuli in cortical pain modulatory regions such as the PFC and hypothalamus. Furthermore, we aim to determine if any activation differences are associated with altered connectivity with brainstem pain modulatory regions, namely the PAG. We also hypothesize that migraineurs will show altered functional connectivity between higher cortical brain regions involved in pain modulation and the PAG, in particular during the 24-hour period immediately prior to a migraine attack.

Methods

Subjects:

Twenty-five subjects with episodic migraine (6 males, mean±SEM age: 29.6±2.0 years, range 19-54) and 29 pain-free controls (10 males, mean±SEM age: 26.4±1.4 years, range 19-57) were recruited for the study. Migraine subjects were diagnosed according to the criteria set by the International Classification of Headache Disorders (ICHD), 3rd edition, sections 1.1 and 1.2 (18). Four migraineurs reported experiencing aura with their migraines, and the remaining 21 reported no aura. Of the 25 migraineurs, 20 were placed into an *interictal* group as they were scanned during the interictal period, i.e. at least 72 hours after and 24 hours prior to a migraine attack. Of the 25 migraineurs, 7 migraineurs were placed into an *immediately prior to migraine* group since they were scanned during the 24-hour period immediately before a migraine. Two migraineurs were scanned during both an interictal and immediately prior to a migraine phase. There were no significant differences in age (*t*-test; $p>0.05$), or gender composition (X^2 test, $p>0.05$) between the three groups.

All migraine subjects indicated the pain intensity (6-point visual analog scale; 0=no pain, 5=most intense imaginable pain) and drew the facial distribution of pain they commonly experienced during a migraine attack. Additionally, each subject described the qualities of their migraines and indicated any current treatments used to prevent or abort a migraine once initiated. Exclusion criteria for controls were the presence of any current pain or chronic pain condition, current use of analgesics, and any neurological disorder. Exclusion criteria for migraineurs were any other pain condition other than migraine or any other neurological disorder. Informed written consent was obtained for all procedures according to the Declaration of Helsinki seventh revision and local Institutional Human Research Ethics Committees approved the study. Data

from several subjects used in this investigation have been used in previous investigations (5, 17, 19-21).

MRI acquisition:

In all control subjects, before entering the magnetic resonance imaging (MRI) scanner, a 3×3 cm MRI-compatible thermode (Medoc, Ramat Yishai, Israel) was placed on the right side of the corner of the mouth covering the upper and lower lips. In migraineurs, the thermode was also placed on the right corner of the mouth except for 4 migraineurs in which it was placed on the left side, since they were the only migraineurs that most commonly experienced headaches on the left side. A temperature that evoked moderate pain ratings was determined for each individual subject with a Thermal Sensory Analyzer (TSA-II, Medoc), from a resting temperature of 32^{o}C to temperatures at 0.5^{o}C intervals between 44^{o}C and 49^{o}C. Temperatures were randomly applied in 15 second intervals for 10 seconds during which each subject rated the pain intensity using a 10 point Computerised Visual Analog Scale (CoVAS, Medoc; 0=no pain, 10=worst imaginable pain). The temperature at which individuals indicated a pain intensity rating of approximately 6 out of 10, was used for the remainder of the experiment.

All subjects then lay supine on the bed of a 3T MRI scanner (Philips, Achieva) with their head immobilized in a 32-channel head coil. With each subject relaxed and at rest, a high-resolution 3D T1-weighted anatomical image set covering the entire brain was collected (turbo field echo; field of view 250×250mm, raw voxel size 0.87mm^3, repetition time 5600ms, echo time 2.5ms, flip angle 8^{o}). Following this, a series of 140 gradient-echo echo planar functional MRI image volumes with blood oxygen level-dependent contrast was collected with each image volume covering the entire brain

(38 axial slices, repetition time 2500ms, raw voxel size 1.5×1.5×4.0mm thick). During this functional magnetic resonance imaging (fMRI) scan, following a 30-volume baseline period, 8 noxious thermal stimuli were delivered (Figure 1A). Each noxious stimulus was delivered for 15 seconds (including ramp up and down periods of 2.5 seconds each), followed by a 15 second baseline (32°C) period. During each period of noxious stimulation, the subject was asked to rate the pain intensity online using the CoVAS.

MRI image preprocessing:

In the 4 migraineurs in whom the thermode was placed on the left side of the mouth (2 scanned during interictal phase, 2 were scanned during both interictal and immediately prior to migraine phases), their MRI images were reflected in the X plane so that in all subjects the right side was ipsilateral to the delivered noxious thermal stimulus. Using SPM12 (22) and custom software, fMRI images were slice-timing corrected, motion corrected and the effect of motion on signal intensity was modelled and removed using LMRP detrending. Physiological (i.e. cardiovascular and respiratory) noise was then modelled and removed using the DRIFTER toolbox (23) and the images were then linear detrended to remove global signal intensity drifts. Each subject's fMRI image set was co-registered to their own T1-weighted anatomical image. The T1 images were then spatially normalized to the Montreal Neurological Institute (MNI) template and the normalization parameters were applied to the fMRI images to place them in MNI space. Finally, the wholebrain images were smoothed using a 6mm full-width half maximum (FWHM) Gaussian filter. In addition, prior to spatial normalization, using brainstem-specific isolation software (SUIT toolbox in SPM12) (24), a mask of the brainstem was created for each subject for both the T1 and fMRI image sets. Using these masks, the brainstem of the T1 and fMRI image

sets were isolated and then spatially normalized to the SUIT brainstem template in MNI space. These brainstem-only image sets were then spatially smoothed using a 3mm FWHM Gaussian filter. A small smoothing kernel was used to maintain spatial accuracy in small brainstem sites.

Acute pain related signal intensity change analysis:

Changes in signal intensity during the 8 noxious stimuli were determined using a repeated box-car model convolved with a canonical haemodynamic response function. Since we have already investigated changes within the brainstem (5), we will assess only those changes above the brainstem using the wholebrain images (cortical/subcortical images). Significant differences between the control group and those migraineurs scanned during the interictal phase (n=20) were determined using a two-group random effects analysis in SPM12 ($p<0.05$, false discovery rate [FDR] (25) corrected for multiple comparisons, minimum cluster size of 10 contiguous voxels, age and gender as nuisance variables). In addition, significant differences between controls and migraineurs scanned during the 24-hour period immediately prior to a migraine headache (n=7) were also determined using a two-group random effects analysis ($p<0.05$, FDR corrected, minimum cluster size 10, age and gender as nuisance variables). Significant clusters were overlaid onto a mean T1 anatomical and beta values for significant clusters were extracted and the mean±SEM plotted for all three groups (controls, migraine interictal, migraine immediately prior to migraine). If a significant cluster was derived from the control versus interictal analysis, then mean beta values were compared between control and immediately prior to migraine groups for that cluster using two-sample *t*-tests ($p<0.05$, Bonferonni corrected for multiple comparisons). In this instance, differences between controls and interictals were not compared to avoid statistical double-dipping.

Cortical/subcortical whole scan connectivity change analysis:

To assess the potential descending influences onto brainstem pain modulatory circuits, we used four of the clusters that displayed significant differences in the initial acute pain activation analysis (ipsilateral nucleus accumbens [NAc], contralateral ventrolateral prefrontal cortex [vlPFC] and two clusters in the contralateral dorsolateral prefrontal cortex [dlPFC]) and performed two different connectivity-based analyses. *Whole scan connectivity:* for each of these clusters we firstly performed a whole scan functional connectivity analysis. That is, we extracted the mean signal intensity change from the cortical/subcortical image sets for each of the four clusters and then performed a voxel-by-voxel connectivity analysis over the entire fMRI scan, creating four brain maps with each voxel indicating connectivity strength for each subject. Significant differences in whole scan connectivity strengths between controls and interictals and between controls and immediately prior to migraine were determined using two-group random effects analyses ($p<0.05$, FDR corrected, minimum cluster size 10 voxels, age and gender nuisance variables).

Cortical/subcortical pain-related connectivity change analysis:

In addition to whole scan connectivity changes, we assessed pain-related changes in connectivity strengths for each of the four clusters. We used a psychophysiological interaction (PPI) analysis technique in SPM12 which allows for examination of the interaction between the signal covariations of a physiological variable (four seeds) and a psychological variable (noxious orofacial stimulation) (26, 27). The resultant brain maps provide an indicator of the degree and direction to which connectivity changes during the noxious stimulus periods compared with the baseline periods. Significant differences in pain-related changes in connectivity strengths between controls and

241 interictals and between controls and immediately prior to migraine were determined
242 using two-group random effects analyses ($p<0.05$, FDR corrected, minimum cluster
243 size 10 voxels, age and gender nuisance variables).
244
245 Dorsolateral PFC and hypothalamus brainstem specific connectivity change analysis:
246 The cortical/subcortical whole scan and pain-related changes in connectivity analysis
247 revealed that only one of the four clusters, the dlPFC [at Z level +44], showed
248 significant differences between controls and migraineurs. Since this brain region has
249 been heavily implicated in pain modulation (28, 29), we focussed our subsequent
250 analysis on this dlPFC region. Furthermore, it is thought that the dlPFC modulates the
251 brainstem directly or via projections to the hypothalamus (29) and our whole scan
252 connectivity analysis revealed a significant change in dlPFC connectivity with the
253 hypothalamus in migraineurs. Given this we determined whether changes in whole
254 scan and pain-related changes in connectivity strengths between the dlPFC and
255 brainstem and between the hypothalamus and brainstem were altered in migraineurs.
256 Using the brainstem specific fMRI images, we used the dlPFC and hypothalamic
257 seeds to assess whole scan and pain-related changes in connectivity in the same
258 analysis procedures described above.
259
260 Significant differences in wholebrain and pain-related changes in brainstem
261 connectivity between the migraine groups and controls were determined using two-
262 group random effects analyses ($p<0.001$, uncorrected, minimum cluster size 10
263 voxels, age and gender nuisance variables). To reduce the likelihood for Type 1 errors
264 we performed cluster level correction for multiple comparisons. Significant clusters
265 were overlaid onto a standard brainstem template and mean±SEM connectivity
266 strengths plotted for each cluster for each group. If a significant cluster was derived

from the control versus interictal analysis, then connectivity strength values were compared between control and immediately prior to migraine groups for that cluster using two-sample *t*-tests ($p<0.05$). The location of brainstem clusters was identified using the Atlas of the Human Brainstem (30) and the Duvernoy Brainstem Atlas (31).

Results

Migraine characteristics:

Using a self-report questionnaire, of the 25 migraineurs, 11 reported that their headaches occurred most commonly on the right side, while 4 reported more on the left and the remaining 10 reported that they would occur on either side (see Table 1 for migraineur characteristics). Migraine subjects most frequently described their migraine pain as “throbbing,” “sharp,” and/or “pulsating” in nature and indicated that “stress,” “lack of sleep,” and/or “bright light” most often triggered their migraine attacks. The mean estimated frequency of migraine attacks was 16.4±1.9 per year, mean length of time since the onset of migraine attacks (years suffering) 15.4±2.3 years, and mean pain intensity of migraines 3.7±0.2 on a 6-point visual analog scale. Although 16 of the 25 migraineurs were taking some form of daily medication (mostly the oral contraceptive pill; 10 migraineurs), none of the migraine subjects were taking prophylactic medication prescribed for migraine.

Pain ratings:

The overall pain intensity ratings during the 8 brief noxious heat stimuli were similar in all 3 groups (mean±SEM VAS: controls 5.4±0.4; interictal 4.5±0.5; immediately prior to migraine 4.9±0.7; two-tailed *t*-test, all $p>0.05$). In addition, there was also no significant difference in the applied thermode temperature used to evoke these pain

levels between groups (mean temperature: controls 47.7±0.2°C; interictal 48±0.2°C; immediately prior to migraine 47.9±0.3°C; Figure 1).

Acute pain related signal intensity changes:

Across all subjects, acute noxious stimuli evoked significant signal intensity increases in a number of brain regions, including the insula, cingulate cortex, primary and secondary somatosensory cortices and decreases in the medial prefrontal and posterior parietal cortices and in the precuneus. Analysis of acute pain evoked changes in activation between groups revealed no significant differences between controls and migraineurs during the interictal phase. However, comparison of migraineurs immediately prior to a migraine revealed significant increases in a number of brain regions including the ipsilateral NAc, the contralateral vlPFC and two clusters in the dlPFC as well as the posterior parietal cortex and temporal cortex (Figure 2, Table 2). Extraction of the magnitude of signal changes (beta values) also revealed that changes in signal within the four clusters: ipsilateral NAc, the contralateral vlPFC and two clusters in the dlPFC, did not change during the interictal period compared with controls (mean±SEM beta values in controls, interictal, immediately prior to migraine: NAc 0.01±0.04, 0.01±0.07, p=0.99, 0.51±0.17, p<0.001; vlPFC -0.06±0.06, 0.04±0.12, p=0.43, 0.43±11, p=0.001; dlPFC[Z level +28] -0.13±0.06, 0.04±0.11, p=0.14, 0.35±0.08, p<0.001; dlPFC[Z level +44] -0.29±0.07, -0.22±0.15, p=0.64, 0.24±0.09, p=0.001; all control versus interictal p>0.05).

Table 2. Montreal Neurological Institute (MNI) coordinates, cluster size and t-score for regions with greater signal intensity changes.

Brain region	MNI Co-ordinate			cluster size	t-score
	x	y	z		
ventrolateral prefrontal cortex	-46	40	-4	16	3.92
	-52	24	10	29	3.76
posterior parietal cortex	-66	-48	-2	75	5.27
temporal cortex	-62	-20	-6	22	3.91
dorsolateral prefrontal cortex	-48	24	28	55	3.88
	-48	10	44	20	3.77
nucleus accumbens	10	6	-10	23	3.53

Cortical/subcortical whole scan connectivity changes:

Whole scan connectivity analysis revealed no significant differences between the control and interictal groups for any of the four clusters, i.e. NAc, vlPFC, dlPFC[Z+28], dlPFC[Z+44]. Similarly, comparison of whole scan connectivity between control and immediately prior to migraine groups revealed no significant differences for the NAc, vlPFC or dlPFC[Z+28] clusters, however significant differences did occur in a number of brain regions for the dlPFC[Z+44] cluster. Significantly reduced whole scan dlPFC connectivity strengths occurred in the contralateral orbitofrontal cortex (OFC), putamen, ventroposterior (VP) thalamus, hippocampus, dlPFC and the ipsilateral putamen, hypothalamus, primary motor cortex (M1) and posterior parietal cortex (Figure 3, Table 3). Extraction of the magnitude of connectivity strength also revealed that changes in whole scan dlPFC connectivity within these clusters decreased significantly during the interictal phase in migraineurs compared with controls in the contralateral putamen (mean±SEM connectivity strength values in controls, interictals, immediately prior to migraine: 0.13±0.01, 0.05±0.02, p=0.005, -0.05±0.01, p<0.001),

342 contralateral dlPFC (0.19±0.02, 0.07±0.03, p=0.002, -0.01±0.04, p<0.001),
343 contralateral OFC (0.22±0.02, 0.12±0.02, p=0.001, 0.03±0.03, p<0.001), and
344 ipsilateral M1 (0.17±0.02, 0.07±0.03, p=0.005, -0.03±0.05, p<0.001), but did not
345 significantly change during the interictal period in the contralateral VP thalamus
346 (0.14±0.02, 0.10±0.02, p=0.24, -0.02±0.02, p=0.001), ipsilateral putamen (0.09±0.02,
347 0.07±0.02, p=0.35, -0.09±0.02, p<0.001), or the ipsilateral hypothalamus (0.08±0.01,
348 0.06±0.02, p=0.39, -0.04±0.01, p<0.001). Furthermore, in the contralateral VP
349 thalamus, ipsilateral putamen, hypothalamus and OFC, whole scan connectivity
350 strength values were significantly decreased immediately prior to migraine compared
351 with the interictal phase in migraineurs. In no region was whole scan connectivity
352 strengths increased in migraineurs compared with controls.
353
354 <u>Cortical/subcortical pain-related connectivity changes:</u>
355 Analysis of noxious stimulus related pain-related changes in connectivity strengths
356 (PPI analysis) revealed no significant differences between the control and interictal
357 groups or between the control and immediately prior to migraine groups for any of the
358 four clusters. All control versus immediately prior to migraine p>0.05 and all control
359 versus interictal p>0.05.
360
361 <u>Dorsolateral PFC and hypothalamus brainstem specific connectivity changes:</u>
362 Given that it is known that the dlPFC can modulate pain by either direct descending
363 projections to the brainstem or indirectly via the hypothalamus, we determined whether
364 there were any whole scan or pain-related changes in noxious-stimulus related
365 connectivity changes between the dlPFC[Z+44] and the brainstem as well as between
366 the hypothalamus (cluster derived from whole scan dlPFC analysis) and the
367 brainstem. Comparison of control with immediately prior to migraine groups revealed

368 no significant whole scan connectivity differences between either the dlPFC[Z+44] or
369 hypothalamus. In addition, whilst there were also no significant differences in
370 brainstem whole scan connectivity between the hypothalamus and brainstem during
371 the interictal phase, the dlPFC[Z+44] displayed significantly reduced whole scan
372 connectivity with a discrete region of the rostral ventrolateral medulla (mean±SEM
373 connectivity strength values in controls, interictals, immediately prior to migraine:
374 0.06±0.02, -0.10±0.05, p<0.001, -0.02±0.03, p=0.11)(Table 3).
375
376 In striking contrast, whilst comparison of control and interictal migraine groups
377 revealed no significant differences in brainstem pain-related changes in connectivity
378 for either the dlPFC or hypothalamus, comparison of controls with immediately prior
379 to migraine group, revealed significant pain-related changes in connectivity
380 differences within multiple brainstem sites. Significantly reduced dlPFC[Z+44]
381 changes in pain-related connectivity strengths occurred in the regions of the
382 contralateral dorsolateral pons (dlPons), the dorsomedial pons (dmPons) spreading
383 into the ipsilateral dlPons, the ipsilateral SpV and a larger cluster centered in the region
384 of the subnucleus reticularis dorsalis (SRD) and extending to encompass the
385 contralateral SpV and rostral ventromedial medulla (RVM)(Figure 4A, Table 3).
386 Extraction of the magnitude of pain-related connectivity strength changes also
387 revealed that these changes were restricted to the period immediately prior to migraine
388 and did not change during the interictal phase relative to controls (mean±SEM PPI in
389 controls, interictals, immediately prior to migraine: ipsilateral dlPons 0.05±0.04, -
390 0.08±0.10, p=0.22, -1.12±0.19, p<0.001; dmPons 0.05±0.04, -0.05±0.08, p=0.23, -
391 1.69±0.58, p<0.001; ipsilateral SpV 0.13±0.04, -0.14±0.12, -0.97±0.31, p<0.001;
392 SRD/SpV/RVM 0.10±0.03, -0.06±0.09, p=0.07, -1.12±0.22, p<0.001).
393

Pain-related changes in connectivity analysis of the right hypothalamus also revealed significantly reduced pain-related connectivity within the brainstem although in a more restricted pattern. Whilst there were no significant differences between controls and interictal migraine groups, significantly reduced hypothalamus pain-related changes in connectivity occurred during the period immediately prior to a migraine in the contralateral midbrain periaqueductal gray matter (PAG), the dlPons bilaterally and in the RVM (Figure 4B, Table 3). Again, extraction of the magnitude of pain-related changes in connectivity strength changes also revealed that these changes were restricted to the period immediately prior to migraine and did not change during the interictal phase relative to controls (mean±SEM PPI in controls, interictals, immediately prior to migraine: contralateral PAG 0.51±0.24, -0.28±0.31, p=0.05, -1.81±0.80, p=0.001; contralateral dlPons 0.08±0.10, -0.25±0.16, p=0.07, -1.46±0.61, p<0.001; ipsilateral dlPons 0.05±0.09, 0.05±0.15, p=0.99, -1.03±0.20, p<0.001; RVM -0.09±0.16, 0.21±0.22, p=0.27, -1.73±0.62, p=0.001).

Discussion

The results of this study demonstrate that in migraineurs, immediately prior to a migraine event, acute orofacial noxious stimuli evoke greater signal changes in cortical and subcortical regions compared with controls, even though the perceived pain intensities are not different. One of these regions, the dlPFC, also displayed decreased whole scan functional connectivity with the hypothalamus and both the dlPFC and hypothalamus displayed reduced pain-related changes in connectivity with brainstem pain modulatory regions. Importantly, these connectivity strength decreases in migraineurs were restricted to the period immediately prior to a migraine attack. These results indicate that immediately prior to a migraine, brainstem pain modulating

419 circuitry control is modulated by the cortex, potentially influencing the on-going activity
420 and/or sensitivity of the brainstem region receiving orofacial afferent drive.
421
422 Immediately prior to a migraine, migraineurs demonstrated significantly greater acute
423 pain evoked signal intensity changes compared with controls in four regions, the
424 ipsilateral NAc, contralateral vlPFC and two clusters in the contralateral dlPFC.
425 Interestingly, these differences occurred even though on average, perceived pain
426 intensities were similar in controls and migraineurs throughout the migraine cycle. Pain
427 induced activations of the NAc (32, 33), vlPFC (34) and the dlPFC (35) have been
428 demonstrated in previous studies. The NAc is associated with the reward system and
429 survival behaviors that reduce the possibility of injury or damage signaled by pain are
430 negatively reinforced (36). In experimental animal studies, analgesic responses can
431 be evoked by injections of morphine into either the PAG or NAc (37, 38) and we have
432 previously shown in humans that the NAc is involved in conditional pain modulation
433 (CPM) analgesia (39). Although the NAc receives input from the PFC and projects
434 indirectly to the PAG (40), we found no differences in either whole scan or pain-related
435 changes in connectivity between the NAc and other brain regions. Similarly, the vlPFC
436 also displayed significantly greater activation during acute noxious stimuli in
437 migraineurs but no difference in whole scan or pain-related changes in connectivity.
438 Pain that is controllable evokes greater vlPFC activation compared to pain that is not
439 controllable and vlPFC activation occurs when individuals are instructed to use a
440 reappraisal strategy to emotionally disengage from a threatening stimulus (41, 42).
441 These reports raise the prospect that in our study, migraineurs may be processing the
442 perceived control over the acute pain experience or another aspect of pain other than
443 being involved in descending modulatory control.
444

In striking contrast to the NAc and vlPFC, the dlPFC displayed significant differences in signal intensity and both whole scan and pain-related changes in connectivity, specifically during the phase immediately prior to a migraine attack. While the dlPFC is typically known for its role in several brain networks such as cognitive processes and working memory (43-45), it has also been established as a key region involved in pain processing and pain modulation (28, 46). It has been proposed that this region may exert active control on pain perception through modulation of corticosubcortical and corticocortical pathways (28). Previous fMRI migraine studies have demonstrated increased activation of the dlPFC during pain (35) and we have previously shown that dlPFC activation and connectivity strength with the brainstem is associated with CPM analgesia (39). In addition, a recent study reported decreased resting state functional connectivity between the dlPFC and PAG in migraineurs, although this study only investigated the interictal phase of migraine (47).

We found that increased activation of the dlPFC in migraineurs immediately prior to a migraine was associated with reduced whole scan connectivity with other pain processing regions such as the VP thalamus, orbitofrontal cortex and also the hypothalamus. The connectivity changes with the hypothalamus were of particular interest since our original hypothesis was that the hypothalamus would be involved in modulating the overall sensitivity of the brainstem. Whilst we did not find differences in hypothalamic signal intensity changes during noxious stimuli in migraineurs, the reduced dlPFC-hypothalamus whole scan connectivity suggests altered function of this pathway in migraineurs. The decrease in hypothalamic connectivity was located in the same lateral hypothalamic region in which we have previously shown significantly reduced on-going blood flow in migraineurs, specifically during the period immediately prior to a migraine attack (17). Experimental animal tract tracing

investigations have shown that the PAG, in particular the ventrolateral PAG column, receives projections from the lateral hypothalamus (48) and activation of this hypothalamic region can produce analgesia, likely mediated by the PAG (49).

The hypothalamus has been implicated as a critical region in migraine initiation and maintenance through its strong cortical connections and exertion over subcortical regions involved in descending pain modulation (9, 50). Consistent with this idea, we found reduced dlPFC-hypothalamus whole scan connectivity and reduced pain-related changes in connectivity between the lateral hypothalamus and the PAG, dlPons and RVM immediately prior to migraine. Interestingly, whilst we did not find altered whole scan connectivity between the lateral hypothalamus and these brainstem sites, in our previous investigation we reported significantly reduced resting state connectivity between the lateral hypothalamus and these brainstem sites (17). This difference is likely due to the fact that the “whole scan” connectivity reported in this study was derived from a scan during a series of noxious stimuli and subjects were aware prior to the scan that noxious stimuli were to be administered. It may be that knowing that noxious stimuli are about to be administered, significantly changes hypothalamus-brainstem integration in migraineurs only in the period immediately prior to a migraine attack.

It is well-established that the PAG modulates incoming noxious inputs at the SpV via a projection with the RVM (9, 51). Within the RVM, distinct populations of neurons termed “off” and “on” cells can inhibit or facilitate neurotransmission at the SpV (7, 52) and in pain-free controls the balance between these cells regulate nociceptive thresholds (53). In individuals with chronic pain, it has been suggested that there is a shift in pain-modulation system functioning, such that it favors pro-nociception (54). It

497 is possible that in migraineurs, as a migraine approaches the balance of this PAG-
498 RVM-SpV system moves towards one that favors pro-nociception and when an acute
499 noxious stimulus is delivered, the connectivity within this brainstem circuitry is
500 subsequently altered. This is consistent with our previous report of reduced acute-pain
501 connectivity between the RVM and SpV in migraineurs immediately prior to a migraine
502 (5).
503
504 Interestingly, whilst the lateral hypothalamus displayed significant pain-related
505 changes in connectivity with the PAG and RVM, the dlPFC displayed significant
506 changes with the SRD, RVM and SpV, but not the PAG. This suggests that in addition
507 to altered hypothalamic inputs to PAG-RVM-SpV circuitry, SpV function may also be
508 modulated by projections from the dlPFC either directly or via the RVM or the SRD.
509 Experimental animal studies have shown that the SRD is critical for CPM analgesia
510 expression (55) and we have shown in humans that CPM responsiveness is
511 associated with altered activity in the SRD as well as the dlPons (56). We have also
512 shown that reduced resting dlPFC-SRD connectivity strength is associated with
513 greater CPM analgesia (39). It remains unknown if there is a direct neural connection
514 between the SRD and dlPFC in humans and although one rodent tract-tracing
515 investigation did not find a prefrontal-SRD projection (57), another study did (58). We
516 also found altered decreases in pain-related changes in dlPFC-dlPons and
517 hypothalamus-dlPons connectivity in migraineurs immediately prior to a migraine. The
518 dlPons, more specifically the region of the parabrachial nucleus, is a major target of
519 lamina 1 neurons in the dorsal horn, including those receiving inputs from the orofacial
520 region (59, 60) and it has been shown that inhibiting the parabrachial region results in
521 altered on-going activity in the RVM (61).
522

While we are confident in the robustness of our findings, there are several limitations that require consideration. Firstly, alterations in dlPFC function may reflect general processing of noxious stimuli given its role in multiple pain related processes and not the modulation of incoming noxious information as we have proposed. Using connectivity measures we cannot assess the direction of information flow, however, given the strong changes in dlPFC connectivity with brainstem regions with well-established roles in pain modulation, we suggest our interpretation of the results are the most plausible. Secondly, the relatively low spatial resolution of the fMRI images presents difficulties in accurately localizing each cluster to a specific nucleus or region within the brainstem and cortices. We used whole brain and brainstem atlases to identify and define the location of each significant cluster and our clusters overlap with regions demonstrated to be involved in nociceptive transmission within the literature and particularly the descending pain modulatory pathway. Thirdly, given the difficulties involved with capturing the 24-hour phase immediately prior to a migraine, the sample size collected for this phase are smaller than the interictal phase. The use of uncorrected thresholds for the brainstem connectivity analyses also raises the prospect of Type II errors although we used cluster-based correction and a minimum contiguous cluster extent to limit this as a potential issue. Increasing this sample size of the phase immediately prior to a migraine in future studies to validate our findings and improve study power would be highly desirable, although difficult. Finally, analgesic medications have been demonstrated to affect pain modulation in the brain (62), however, only 28% of migraineurs were taking daily analgesic medication. Despite this, we are confident analgesic medication use did not play a significant role in our study, since we found no differences when comparing migraineurs that did and did not take any analgesics.

Conclusions

Overall, our data reveals that immediately prior to a migraine, the dlPFC and hypothalamus exhibit altered descending influence, as evidenced by reduced connectivity strengths, across brainstem structures involved in processing and modulating incoming noxious inputs. These brainstem structures include those in the classic PAG-RVM-SpV analgesic circuit as well as the SRD-SpV loop responsible for CPM analgesia. Curiously, the occurrence of these changes was independent of overall perceived pain intensity and applied stimuli temperature. Our findings support the theory that increased activation of cortical brain regions is reflective of altered SpV modulation by descending circuits which may enable increased on-going neural traffic or external triggers to initiate a migraine and evoke head pain (4). The findings support the idea that central changes in pain circuits may be involved in the generation of a migraine attack.

Abbreviations:

MRI: magnetic resonance imaging; fMRI: functional magnetic resonance imaging; PPI: psychophysiological interaction analysis; NAc: nucleus accumbens; vlPFC: ventrolateral prefrontal cortex; dlPFC: dorsolateral prefrontal cortex.

Declarations:

Ethics approval and consent to participate

This study was approved by the Institutional Human Research Ethics Committee at the University of Sydney and informed written consent was obtained for all participants in accordance with the Declaration of Helsinki.

Consent for publication

Written informed consent for publication was obtained.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by grants 1032072 and 1059182 awarded by the National Health and Medical Research Council of Australia.

Authors' contributions

TM, NM and LH conceived the design of the study. TM analyzed the data and drafted the manuscript. PM customized the software used to analyze data. All authors read, revised and approved the final manuscript

Acknowledgements

The authors wish to thank the many volunteers involved in this study.

606 **References**

607

608 1. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C,
609 Akerman S. Pathophysiology of Migraine: A Disorder of Sensory Processing.
610 Physiological reviews. 2017;97(2):553-622.
611 2. Goadsby PJ. The vascular theory of migraine--a great story wrecked by the
612 facts. Brain : a journal of neurology. 2009;132(Pt 1):6-7.
613 3. Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem
614 mechanisms in migraine. Nature Reviews Neuroscience. 2011;12(10):570-84.
615 4. Burstein R, Noseda R, Borsook D. Migraine: multiple processes, complex
616 pathophysiology. Journal of Neuroscience. 2015;35(17):6619-29.
617 5. Marciszewski KK, Meylakh N, Di Pietro F, Mills EP, Macefield VG, Macey PM,
618 et al. Changes in brainstem pain modulation circuitry function over the migraine cycle.
619 Journal of Neuroscience. 2018;38(49):10479-88.
620 6. Ossipov MH, Dussor GO, Porreca F. Central modulation of pain. Journal of
621 Clinical Investigation. 2010;120(11):3779-87.
622 7. Fields HL, Heinricher MM. Anatomy and physiology of a nociceptive modulatory
623 system. Philosophical transactions of the Royal Society of London Series B, Biological
624 sciences. 1985;308(1136):361-74.
625 8. Heinricher MM, Fields HL. Central nervous system mechanisms of pain
626 modulation. Wall & Melzack's Textbook of Pain. 2013:129-42.
627 9. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal
628 pathways and endorphin circuitry. Annual review of neuroscience. 1984;7:309-38.
629 10. Fields HL, Heinricher MM, Mason P. Neurotransmitters in nociceptive
630 modulatory circuits. Annual review of neuroscience. 1991;14:219-45.

11. Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. Annual review of physiology. 1978;40:217-48.

12. Holstege G. Some anatomical observations on the projections from the hypothalamus to brainstem and spinal cord: an HRP and autoradiographic tracing study in the cat. Journal of Comparative Neurology. 1987;260(1):98-126.

13. Mantyh PW. Connections of midbrain periaqueductal gray in the monkey. I. Ascending efferent projections. Journal of neurophysiology. 1983;49(3):567-81.

14. Ongür D, An X, Price JL. Prefrontal cortical projections to the hypothalamus in macaque monkeys. The Journal of comparative neurology. 1998;401(4):480-505.

15. Schulte LH, May A. The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. Brain : a journal of neurology. 2016;139(Pt 7):1987-93.

16. Schulte LH, Allers A, May A. Hypothalamus as a mediator of chronic migraine: Evidence from high-resolution fMRI. Neurology. 2017;88(21):2011-6.

17. Meylakh N, Marciszewski KK, Di Pietro F, Macefield VG, Macey PM, Henderson LA. Altered regional cerebral blood flow and hypothalamic connectivity immediately prior to a migraine headache. Cephalalgia. 2020;40(5):448-60.

18. ICHD-3β. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38(1):1-211.

19. Marciszewski KK, Meylakh N, Di Pietro F, Macefield VG, Macey PM, Henderson LA. Altered brainstem anatomy in migraine. Cephalalgia. 2018;38(3):476-86.

20. Marciszewski KK, Meylakh N, Di Pietro F, Macefield VG, Macey PM, Henderson LA. Fluctuating Regional Brainstem Diffusion Imaging Measures of Microstructure across the Migraine Cycle. eNeuro. 2019;6(4):1-11.

21. Meylakh N, Marciszewski KK, Di Pietro F, Macefield VG, Macey PM, Henderson LA. Deep in the brain: Changes in subcortical function immediately preceding a migraine attack. Human Brain Mapping 2018;39(6):2651-63.

22. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RS. Statistical parametric maps in functional imaging: a general linear approach. Human Brain Mapping. 1994;2(4):189-210.

23. Särkkä S, Solin A, Nummenmaa A, Vehtari A, Auranen T, Vanni S, et al. Dynamic retrospective filtering of physiological noise in BOLD fMRI: DRIFTER. Neuroimage. 2012;60(2):1517-27.

24. Diedrichsen J. A spatially unbiased atlas template of the human cerebellum. Neuroimage. 2006;33(1):127-38.

25. Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 2002;15(4):870-8.

26. Friston K, Buechel C, Fink G, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. Neuroimage. 1997;6(3):218-29.

27. O'Reilly JX, Woolrich MW, Behrens TE, Smith SM, Johansen-Berg H. Tools of the trade: psychophysiological interactions and functional connectivity. Social Cognitive and Affective Neuroscience. 2012;7(5):604-9.

28. Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain : a journal of neurology. 2003;126(Pt 5):1079-91.

29. Hadjipavlou G, Dunckley P, Behrens TE, Tracey I. Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: a diffusion tensor imaging study in healthy controls. Pain. 2006;123(1-2):169-78.

682 30. Paxinos G, Huang X-F. Atlas of the Human Brainstem. 1st ed. San Diego, CA:
683 Academic Press; 1995.
684 31. Naidich TP, Duvernoy HM, Delman BN, Sorensen AG, Kollias SS, Haacke EM.
685 Duvernoy's atlas of the human brain stem and cerebellum. Vienna: Springer2009.
686 32. Becerra L, Borsook D. Signal valence in the nucleus accumbens to pain onset
687 and offset. European Journal of Pain. 2008;12(7):866-9.
688 33. Aharon I, Becerra L, Chabris CF, Borsook D. Noxious heat induces fMRI
689 activation in two anatomically distinct clusters within the nucleus accumbens.
690 Neuroscience letters. 2006;392(3):159-64.
691 34. Witting N, Kupers RC, Svensson P, Arendt-Nielsen L, Gjedde A, Jensen TS.
692 Experimental brush-evoked allodynia activates posterior parietal cortex. Neurology.
693 2001;57(10):1817-24.
694 35. Schwedt TJ, Chong CD, Chiang CC, Baxter L, Schlaggar BL, Dodick DW.
695 Enhanced pain-induced activity of pain-processing regions in a case-control study of
696 episodic migraine. Cephalalgia. 2014;34(12):947-58.
697 36. Navratilova E, Xie JY, Okun A, Qu C, Eyde N, Ci S, et al. Pain relief produces
698 negative reinforcement through activation of mesolimbic reward-valuation circuitry.
699 Proceedings of the National Academy of Sciences of the United States of America.
700 2012;109(50):20709-13.
701 37. Khalilzadeh E, Saiah GV. The possible mechanisms of analgesia produced by
702 microinjection of morphine into the lateral habenula in the acute model of trigeminal
703 pain in rats. Research in pharmaceutical sciences. 2017;12(3):241-8.
704 38. Ma QP, Shi YS, Han JS. Further studies on interactions between
705 periaqueductal gray, nucleus accumbens and habenula in antinociception. Brain
706 research. 1992;583(1-2):292-5.

707 39. Youssef AM, Macefield VG, Henderson LA. Cortical influences on brainstem
708 circuitry responsible for conditioned pain modulation in humans. Human Brain
709 Mapping. 2016;37(7):2630-44.
710 40. Harris HN, Peng YB. Evidence and explanation for the involvement of the
711 nucleus accumbens in pain processing. Neural regeneration research.
712 2020;15(4):597-605.
713 41. Wiech K, Kalisch R, Weiskopf N, Pleger B, Stephan KE, Dolan RJ. Anterolateral
714 prefrontal cortex mediates the analgesic effect of expected and perceived control over
715 pain. Journal of Neuroscience. 2006;26(44):11501-9.
716 42. Salomons TV, Johnstone T, Backonja MM, Shackman AJ, Davidson RJ.
717 Individual differences in the effects of perceived controllability on pain perception:
718 critical role of the prefrontal cortex. Journal of cognitive neuroscience. 2007;19(6):993-
719 1003.
720 43. Elliott R. Executive functions and their disorders: Imaging in clinical
721 neuroscience. British Medical Bulletin 2003;65(1):49-59.
722 44. Curtis CE, D'Esposito M. Persistent activity in the prefrontal cortex during
723 working memory. Trends in cognitive sciences. 2003;7(9):415-23.
724 45. Hertrich I, Dietrich S, Blum C, Ackermann H. The Role of the Dorsolateral
725 Prefrontal Cortex for Speech and Language Processing. Frontiers in human
726 neuroscience. 2021;15:1-16.
727 46. Ong WY, Stohler CS, Herr DR. Role of the Prefrontal Cortex in Pain Processing.
728 Molecular neurobiology. 2019;56(2):1137-66.
729 47. Chen Z, Chen X, Liu M, Liu S, Ma L, Yu S. Disrupted functional connectivity of
730 periaqueductal gray subregions in episodic migraine. Journal of Headache and Pain.
731 2017;18(1):1-9.

48. Bandler R, Keay KA, Floyd N, Price J. Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain research bulletin. 2000;53(1):95-104.

49. Behbehani MM, Park MR, Clement ME. Interactions between the lateral hypothalamus and the periaqueductal gray. Journal of Neuroscience. 1988;8(8):2780-7.

50. Saper CB. Hypothalamic connections with the cerebral cortex. Progress in brain research. 2000;126:39-48.

51. Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends in neurosciences. 1994;17(9):379-89.

52. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. Brain research reviews. 2009;60(1):214-25.

53. Heinricher MM, Barbaro NM, Fields HL. Putative Nociceptive Modulating Neurons in the Rostral Ventromedial Medulla of the Rat: Firing of On- and Off-Cells Is Related to Nociceptive Responsiveness. Somatosensory & Motor Research. 1989;6(4):427-39.

54. Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. Current Opinion in Supportive Palliative Care. 2014;8(2):143-51.

55. Le Bars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain. 1979;6(3):283-304.

56. Youssef AM, Macefield VG, Henderson LA. Pain inhibits pain; human brainstem mechanisms. Neuroimage. 2016;124(Pt A):54-62.

57. Desbois C, Le Bars D, Villanueva L. Organization of cortical projections to the medullary subnucleus reticularis dorsalis: a retrograde and anterograde tracing study in the rat. The Journal of comparative neurology. 1999;410(2):178-96.

58. Almeida A, Cobos A, Tavares I, Lima D. Brain afferents to the medullary dorsal reticular nucleus: a retrograde and anterograde tracing study in the rat. The European journal of neuroscience. 2002;16(1):81-95.

59. Gauriau C, Bernard JF. Pain pathways and parabrachial circuits in the rat. Experimental physiology. 2002;87(2):251-8.

60. Keay KA, Feil K, Gordon BD, Herbert H, Bandler R. Spinal afferents to functionally distinct periaqueductal gray columns in the rat: an anterograde and retrograde tracing study. The Journal of comparative neurology. 1997;385(2):207-29.

61. Roeder Z, Chen Q, Davis S, Carlson JD, Tupone D, Heinricher MM. Parabrachial complex links pain transmission to descending pain modulation. Pain. 2016;157(12):2697-708.

62. Lueptow LM, Fakira AK, Bobeck EN. The contribution of the descending pain modulatory pathway in opioid tolerance. Frontiers in neuroscience. 2018;12:1-9.

Figure Legends

Figure 1 A) Eight acute noxious thermal stimuli were delivered to the corner of the mouth in controls, migraineurs during the interictal phase and in migraineurs in the 24 hours immediately prior to a migraine headache; **B)** Mean±SEM pain intensity ratings over the 8 noxious stimuli for each group; **C)** Mean±SEM pain intensity ratings for each of the 8 noxious stimuli in each group; **D)** Mean±SEM administered thermode temperatures for each of the three groups. Note there were no significant differences in pain intensity rating or thermode temperatures between any of the groups.

Figure 2: Significant differences in signal intensity changes during 8 noxious thermal stimuli in migraineurs immediately prior to a migraine headache compared with controls. Significant clusters are overlaid onto a mean T1-weighted anatomical image. Slice locations in Montreal Neurological Institute space are indicated to the top right of each slice. Note that signal increase changes were significantly greater in four main regions; the ipsilateral nucleus accumbens, contralateral ventrolateral prefrontal cortex (vlPFC) and two clusters in the dorsolateral prefrontal cortex (dlPFC). Plots of mean (±SEM) beta values (effect sizes) for each of these four clusters revealed that acute orofacial pain evoked significant signal intensity increases in migraineurs only during the 24-hour period immediately prior to migraine, that is signal changes were not different between controls and migraineurs during the interictal phase.

Figure 3: Whole scan connectivity: Significant differences in contralateral dorsolateral prefrontal cortex (dlPFC) whole scan connectivity between controls and migraineurs in the period immediately prior to a migraine headache. Significant clusters are overlaid onto a mean T1-weighted anatomical image. Slice locations in Montreal

Neurological Institute space are indicated to the top right of each slice. The dlPFC seed is shown in the lower right inset. Note that connectivity strengths were significantly reduced in a number of brain regions including the orbitofrontal cortex (OFC), putamen, ventroposterior (VP) thalamus, primary motor cortex (M1) and the hypothalamus. Plots of mean (±SEM) beta values (effect sizes) revealed that whole scan connectivity values decreased significantly in migraineurs only during the 24-hour period immediately prior to migraine, that is, they were not different between controls and migraineurs during the interictal phase.

Figure 4: Pain-related connectivity: Significant differences in **A)** contralateral dorsolateral prefrontal cortex (dlPFC) and **B)** ipsilateral hypothalamus acute pain-evoked changes in connectivity (psychophysiological interaction analysis) between controls and migraineurs in the period immediately prior to a migraine headache. Significant clusters are overlaid onto a mean T1-weighted brainstem template image. Slice locations in Montreal Neurological Institute space are indicated to the top right of each slice. The dlPFC and hypothalamic seeds are shown in the lower right inset. Note that dlPFC pain-related connectivity strengths were significantly reduced in a number of brainstem regions including the dorsomedial pons (dmPons), dorsolateral pons (dlPons), spinal trigeminal nucleus (SpV), and a cluster encompassing the nucleus reticularis dorsalis (SRD)/SpV and rostral ventromedial medulla (RVM). More restricted pain-related hypothalamic connectivity changes occurred in the dlPons, RVM and also in the region of the midbrain periaqueductal gray matter (PAG). Plots of mean (±SEM) beta values (effect sizes) revealed that pain-related changes in connectivity decreased significantly in migraineurs only during the 24-hour period immediately prior to migraine, that is they were not different between controls and migraineurs during the interictal phase.

835 **Table 1.** Migraine subject characteristics. M: male; F: female; B: bilateral; L: left; R: right; OCP:

836 oral contraceptive pill.

Subject	*Age*	*Sex*	*Years suffering*	*Pain side*	*Aura*	*Frequency (per month)*	*Intensity (0-5)*	*Medication taken during migraine*	*Daily medication*
1	31	F	25	R	Y	>3	3-4	paracetamol	-
2	53	M	15	B	N	>3	4	ibuprofen, paracetamol	-
3	24	F	20	B	N	>3	4	ibuprofen, paracetamol	OCP, budesonide /formoterol
4	26	F	12	R	N	2	3-4	ibuprofen	OCP
5	27	F	12	R	Y	1	4	ibuprofen	OCP
6	23	F	4	R	N	>3	4	triptan	OCP, metformin hydrochloride
7	25	F	12	L	N	>3	3	aspirin, rizatriptan	desvenlafaxine
8	21	F	1.5	L	N	>3	3	ibuprofen, paracetamol, codeine	OCP
9	26	F	1	L	N	>3	5	paracetamol	OCP
10	29	F	13	R	N	1	2.5	ibuprofen	zopiclone
11	26	F	5	R	N	1	2	aspirin, codeine, ibuprofen	OCP
12	23	F	6	R	N	1	3-4	ibuprofen	OCP
13	23	F	10	B	N	0.5-1	4	ibuprofen, codeine	OCP
14	46	F	15-20	B	N	1	3	sumatriptan	-
15	41	F	40	B	N	2	4	sumatriptan	-
16	26	M	15	B	N	>3	2	TCE, paracetamol, codeine	-
17	23	M	3-4	B	N	0.5-1	3.5	paracetamol, codeine	-
18	23	M	4-5	B	N	0.5 - 1	4	paracetamol	-
19	55	F	40	R	N	0.5 -1	3-4	sumatriptan	telmisartan
20	26	M	20	R	N	0.5-1	4	metamizole	carbamazepine
21	49	F	30	B	N	0.5-1	5	rizatriptan, paracetamol	-
22	27	M	4	B	N	0.5-1	4	ibuprofen	SSRI
23	28	F	25	R	Y	0.25	5	Ibuprofen, codeine	methylphenidate
24	24	F	13	R	Y	>3	5	TCL, paracetamol, codeine	-
25	19	F	4-5	B	N	>3	3	-	Lexapro, OCP

837

838

839 **Table 3.** Montreal Neurological Institute (MNI) coordinates, cluster size and t-score for
840 regions with reduced whole scan and pain-related connectivity changes.

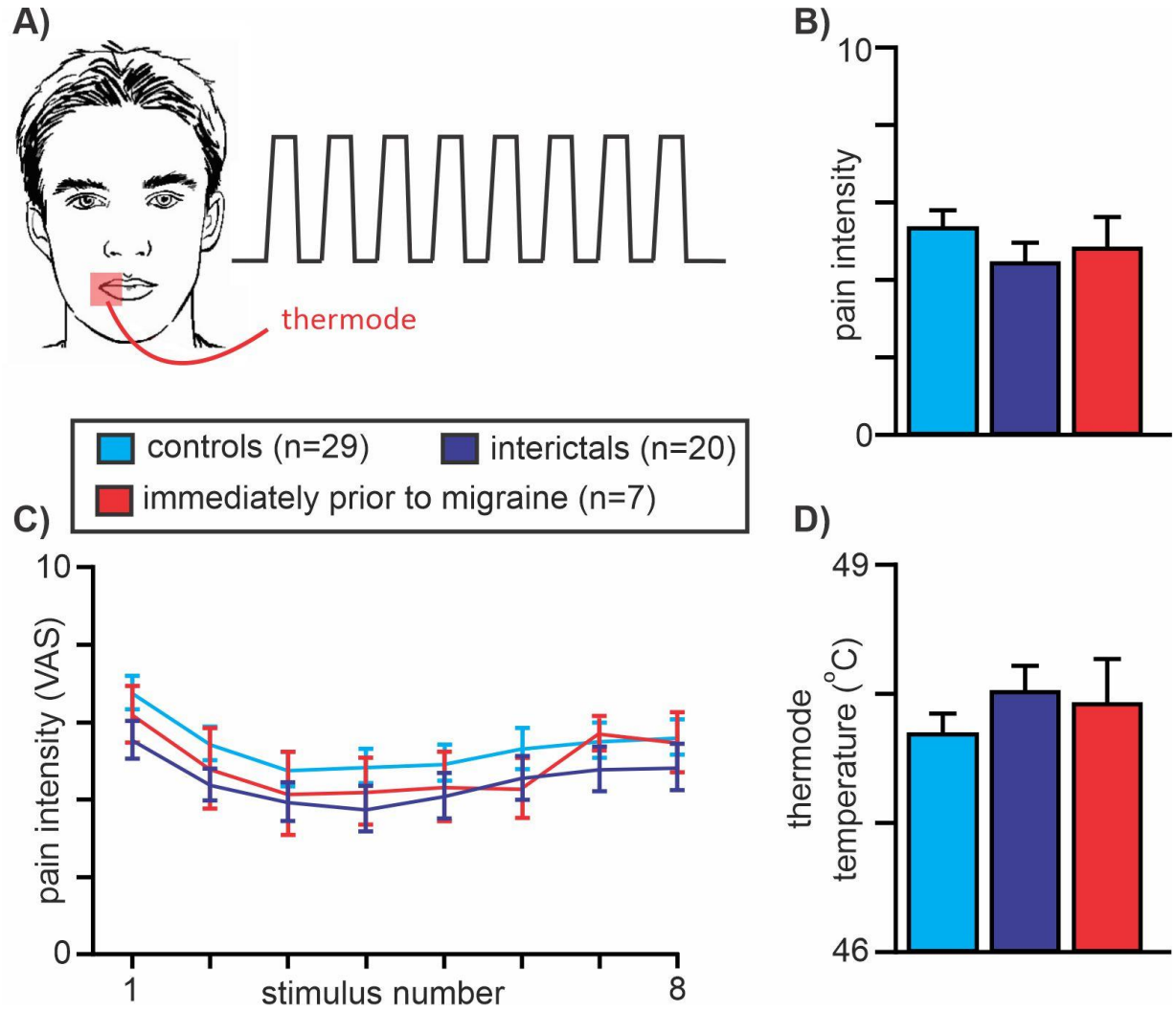
Brain region	**MNI Co-ordinate**			**Cluster size**	**t-score**
	x	y	z		
Dorsolateral prefrontal cortex whole scan cortical/subcortical connectivity					
putamen	-26	2	-14	50	5.63
	-34	-14	-6	92	4.64
posterior parietal cortex	36	-4	-2	71	4.80
	36	-78	-12	340	5.57
hippocampus	-62	-54	-4	37	4.59
hypothalamus	30	-18	-12	45	4.83
orbitofrontal cortex	6	-4	-8	27	4.80
	-40	26	-4	63	4.74
ventral midbrain	-36	36	-16	32	4.55
primary motor cortex	-6	-24	-10	34	4.35
dorsolateral prefrontal cortex	48	-6	32	21	4.22
	-36	14	28	22	4.05
Dorsolateral prefrontal cortex whole scan brainstem connectivity					
rostral ventromedial medulla	-1	-40	-51	12	3.54
Dorsolateral prefrontal cortex pain-related changes in brainstem connectivity					

subnucleus reticularis dorsalis/ spinal trigeminal nucleus/ /rostral ventromedial medulla	-3	-44	-54	141	5.86
spinal trigeminal nucleus	9	-42	-48	35	4.24
dorsomedial pons	13	-36	-38	101	5.34
dorsolateral pons	12	-31	-31	66	4.30
Lateral hypothalamus pain-related changes in brainstem connectivity					
midbrain periaqueductal gray matter	-1	-35	-7	45	3.58
dorsolateral pons	7	-38	-32	45	4.41
	-9	-40	-30	87	3.91
rostral ventromedial medulla	-3	-28	-41	10	4.00

841

842

843 **Figure 1:**



844

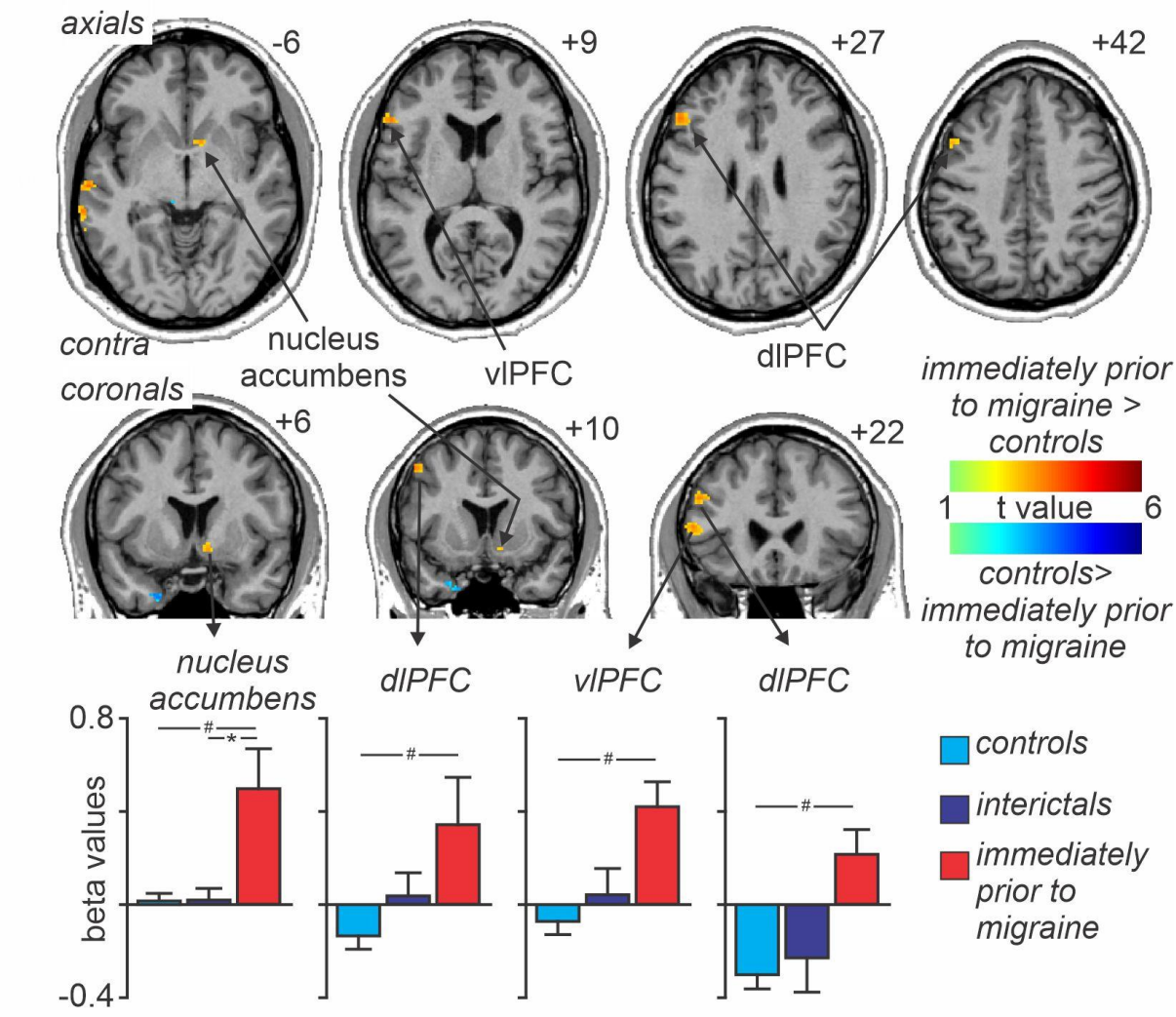
845

846

847

848 **Figure 2:**

849

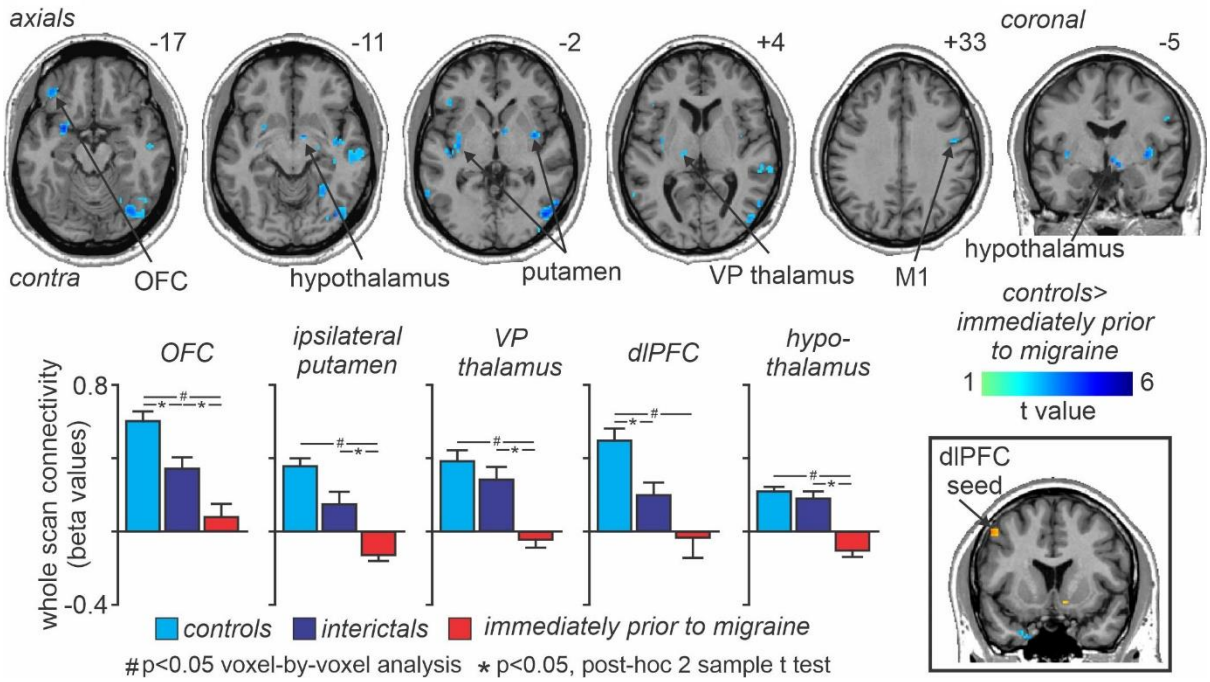


850 # p<0.05 voxel-by-voxel analysis ∗ p<0.05, post-hoc 2 sample t test

851

852 **Figure 3:**

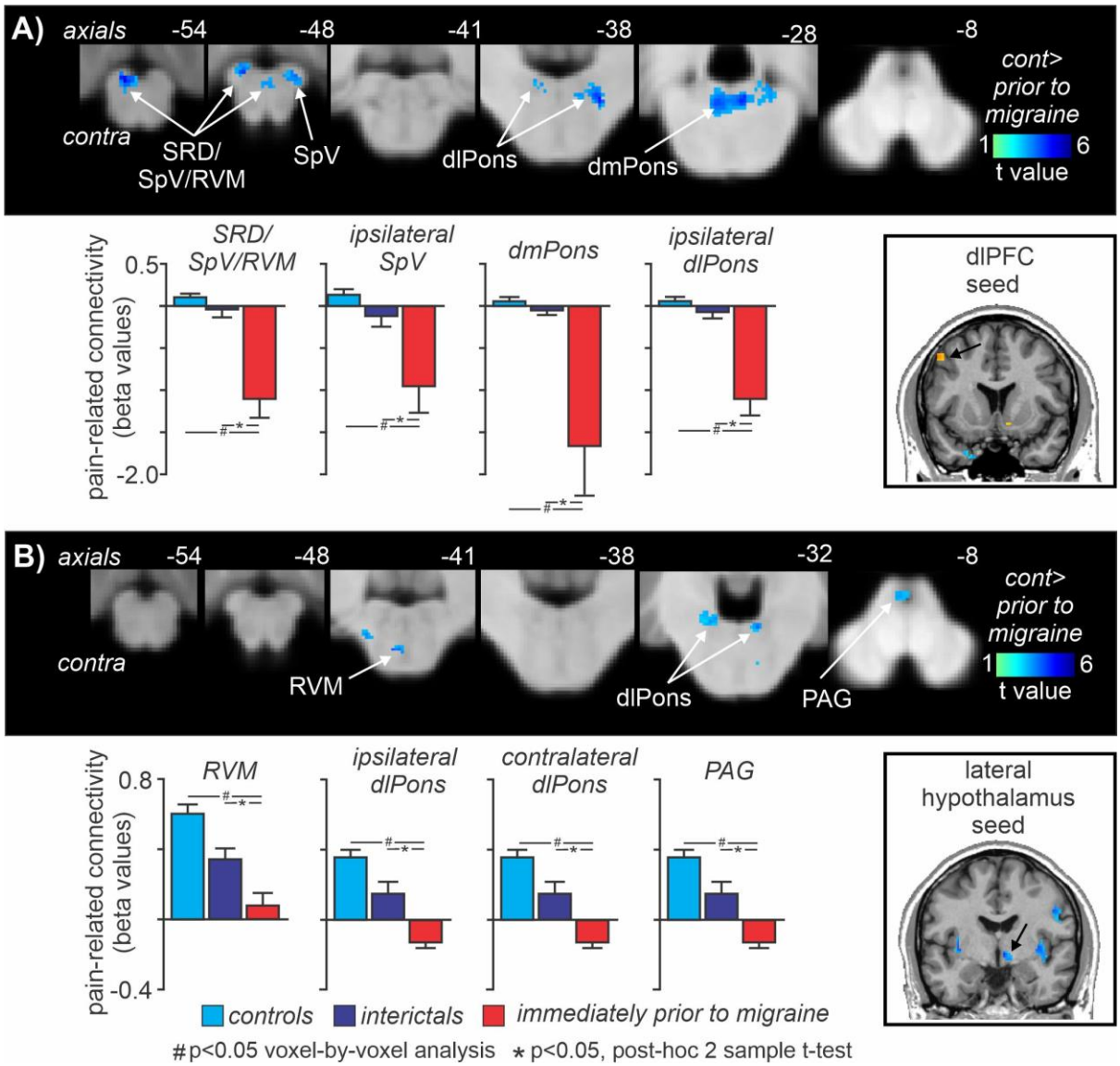
853



854

855

856 **Figure 4:**



857

858